# HTRF assays of IP1, cAMP and pERK pathways employed to study biased signaling of the calcium-sensing receptor
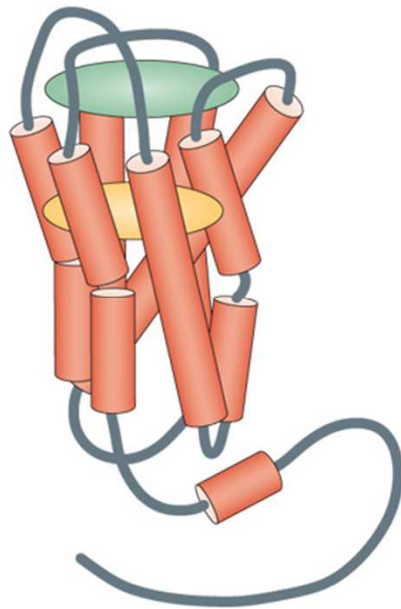
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Molecular Pharmacology Group
Department of Drug Design and Pharmacology
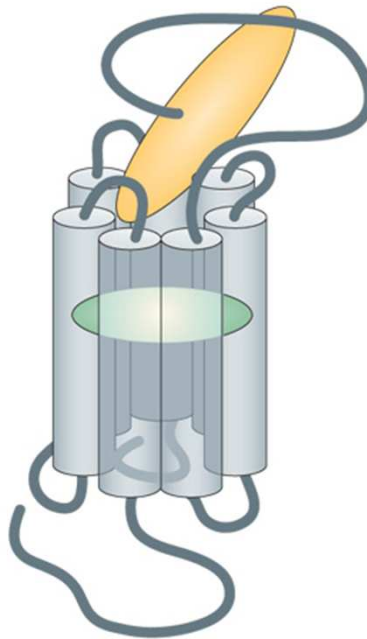www.farma.ku.dk/MolPharm
hbo@sund.ku.dk
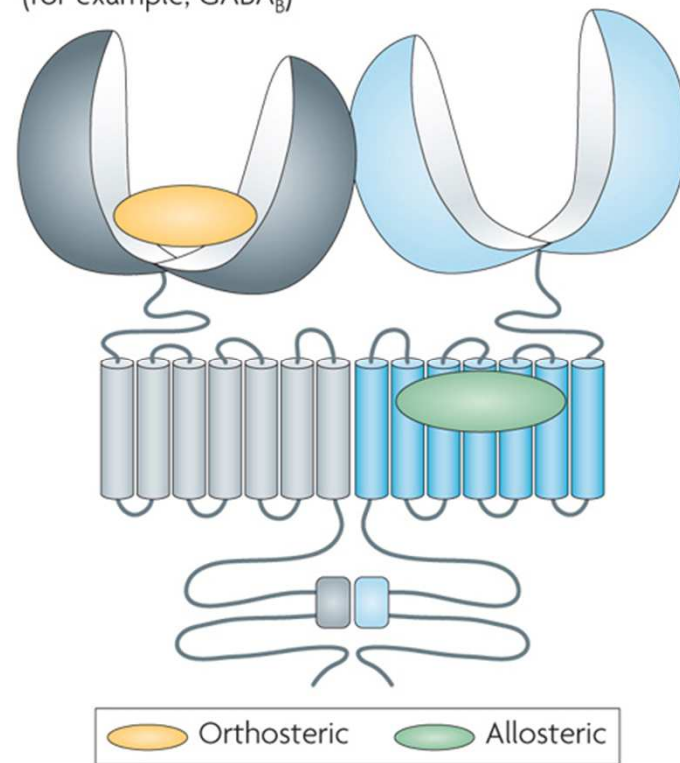
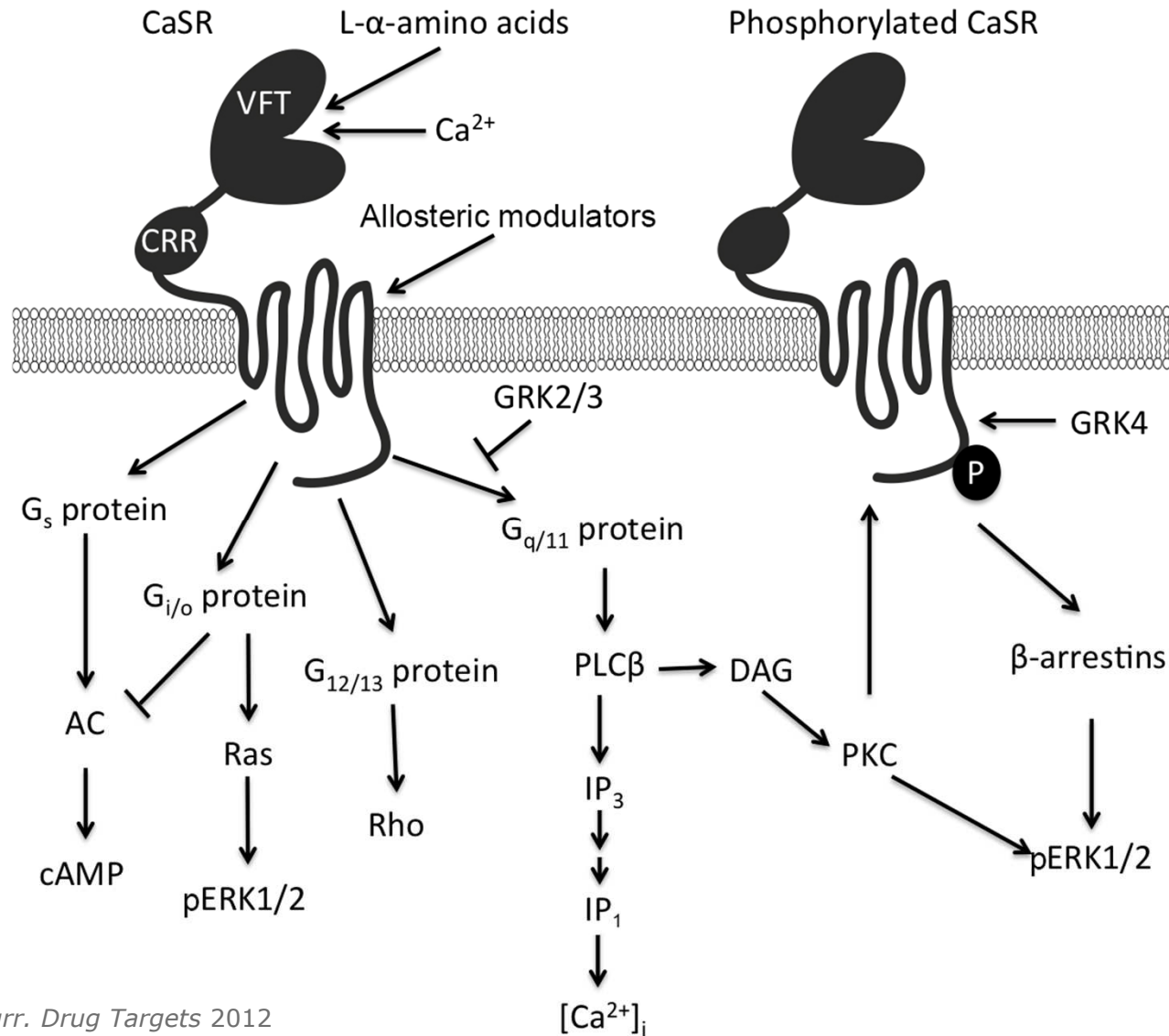# G protein-coupled receptor families/classes



**Class A** (for example, M2 mAChR)
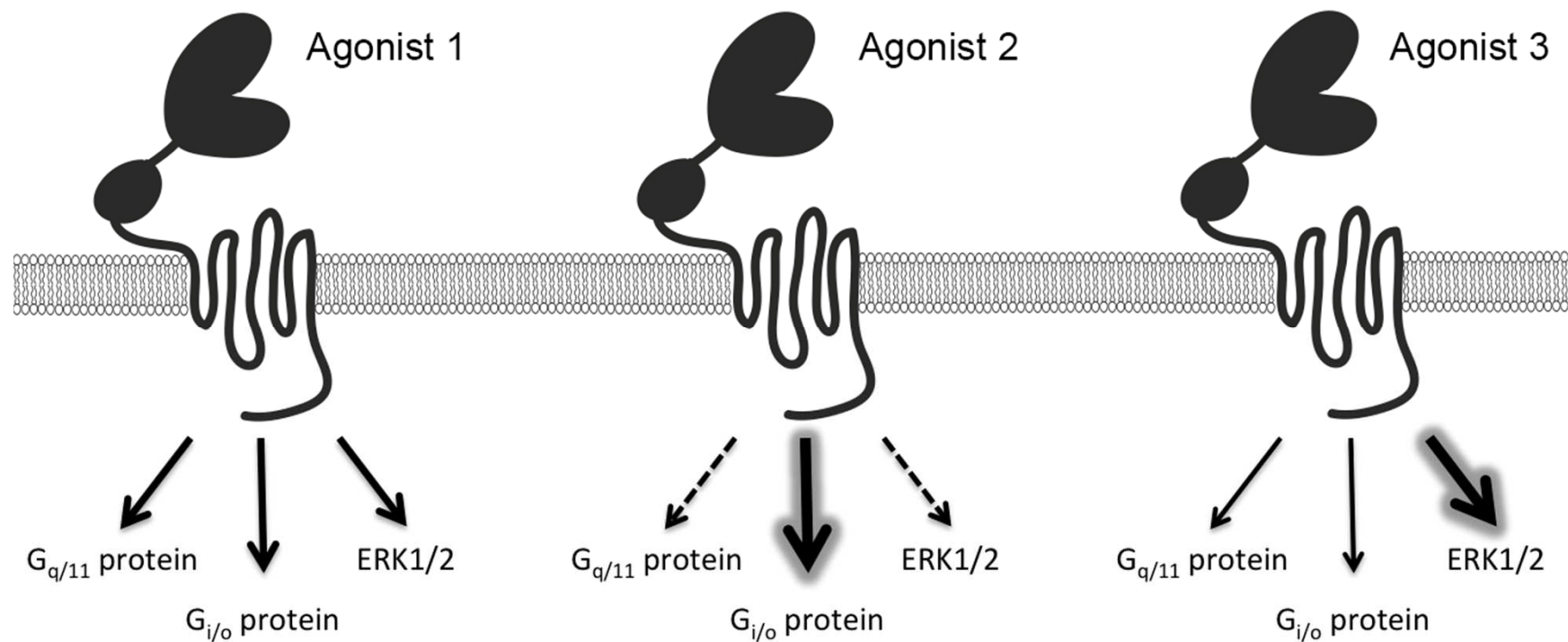
**Class B** (for example, CRF1)

**Class C** (for example, GABA_B)

Orthosteric    Allosteric

# Calcium-sensing receptor (CaSR) signaling pathways
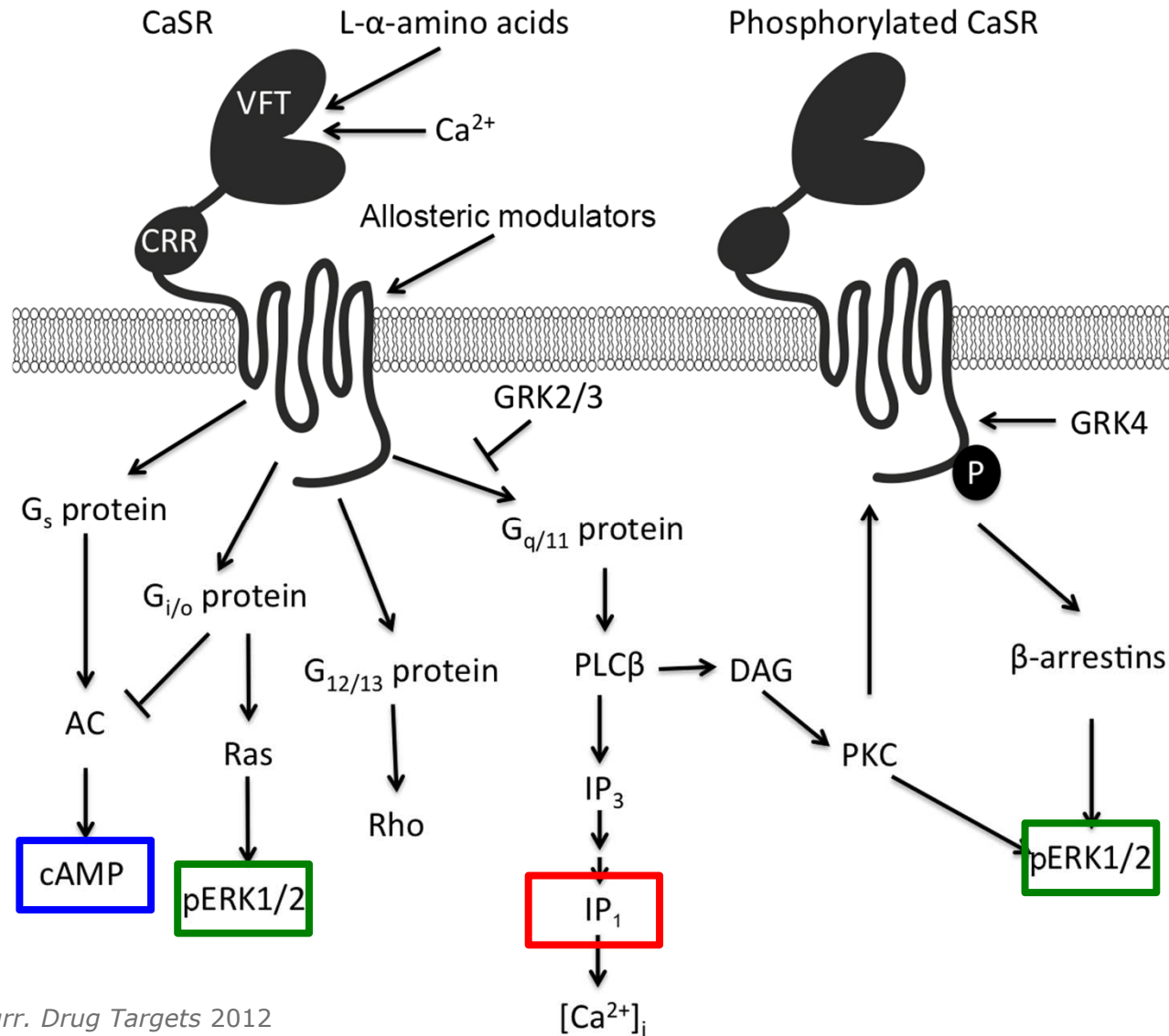


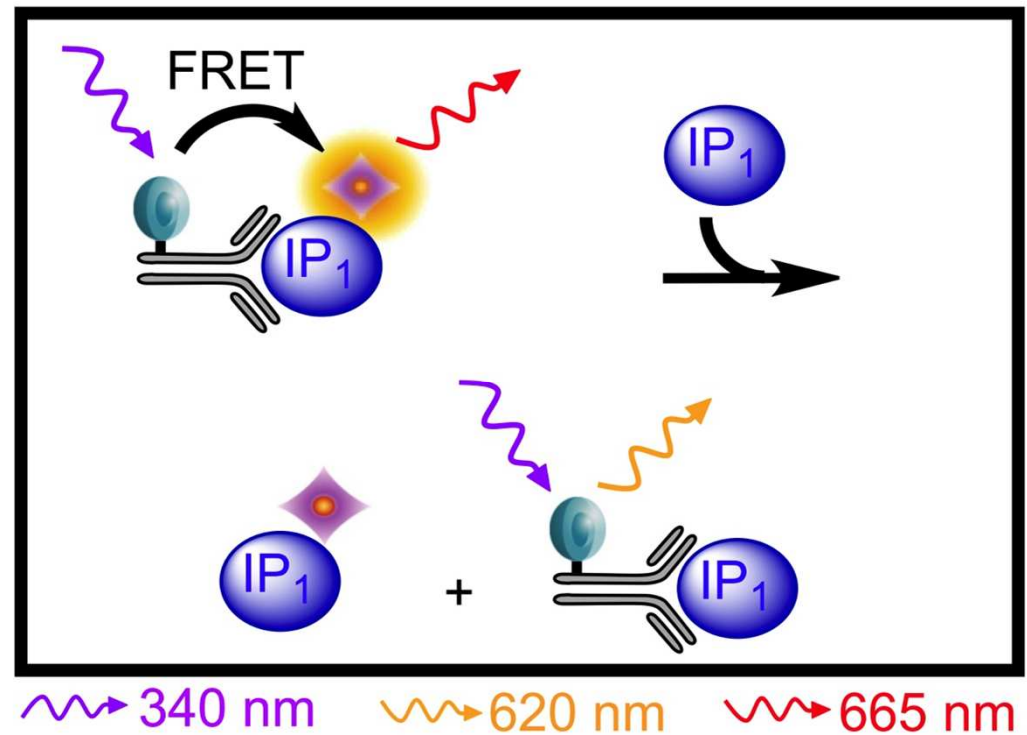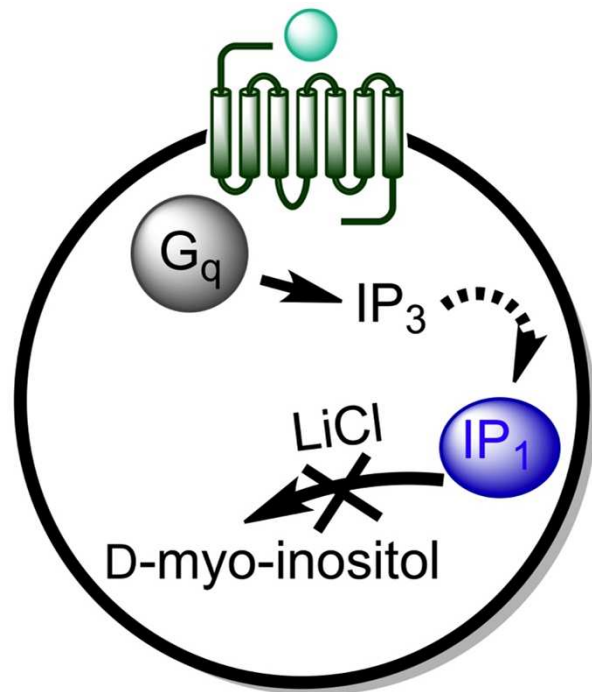Thomsen et al. *Curr. Drug Targets* 2012

# Biased signaling



- Ideally, we would like to measure all possible pathways individually for each ligand.
- Require efficient pharmacological assays.

Thomsen et al. *Curr. Drug Targets* 2012

# Is CaSR signaling biased?



Thomsen et al. *Curr. Drug Targets* 2012

# HTRF assay of IP$_1$ generation
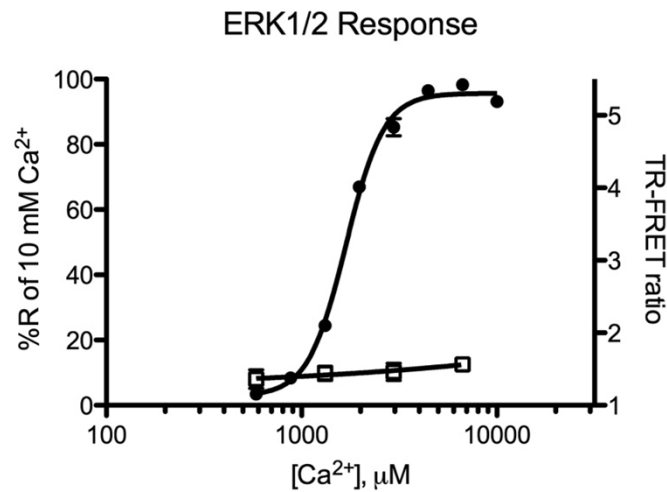


- 384 well format
- HEK293 cells in suspension
- Plates read on EnVision (PerkinElmer)
- cAMP assay function in similar fashion

Nørskov-Lauritsen et al. *Submitted.*

# HTRF assay of ERK1/2 phosporylation
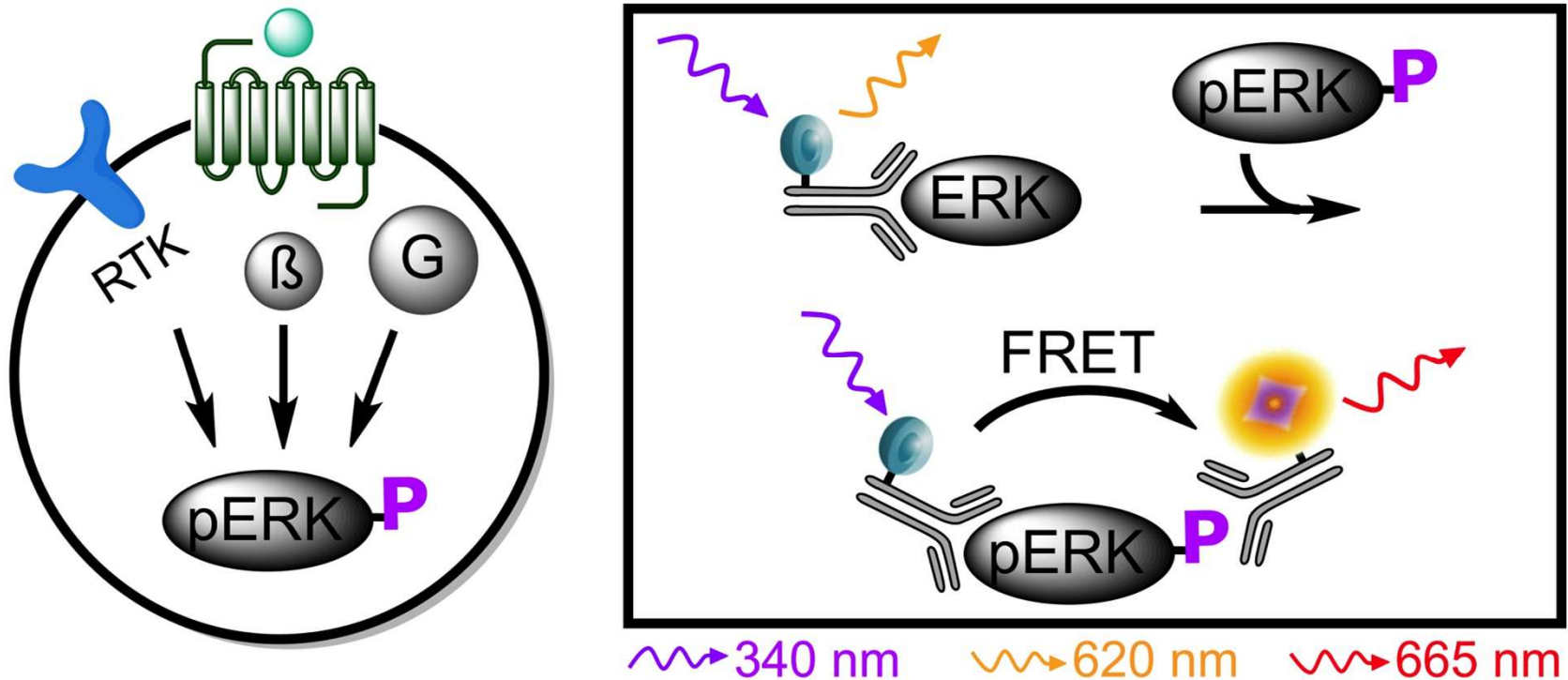


- HEK293 adherent cells in 96 well plates
- transfer supernatant to 384 well HTRF assay plate
- Plates read on EnVision (PerkinElmer)

# Ca$^{2+}$ agonist response
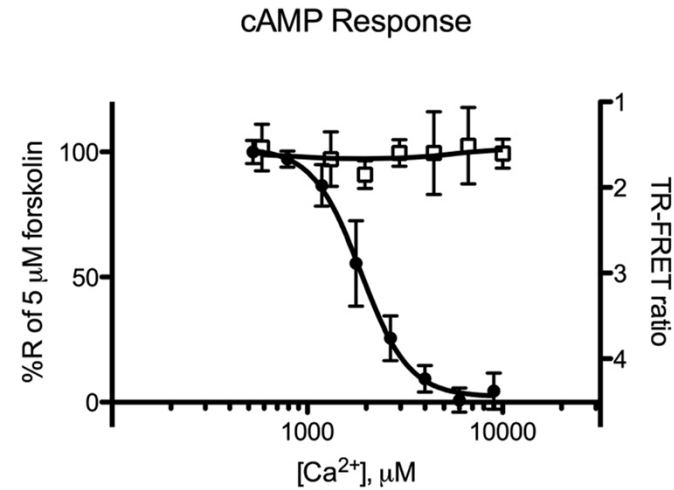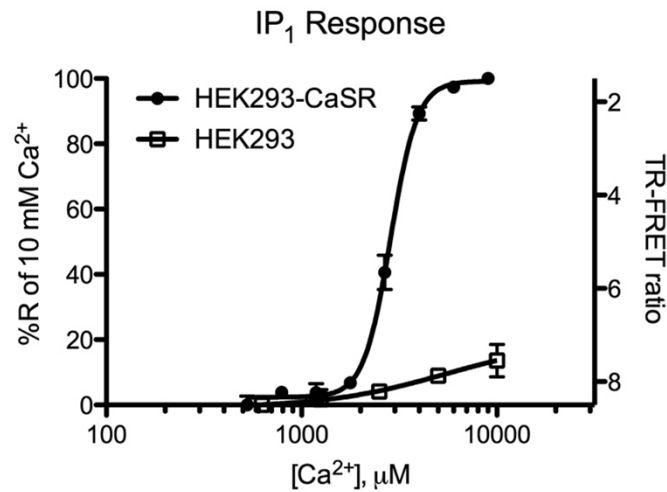


| Pathway/Ligand | Ca$^{2+}$, mM (%) |
|---|---|
| IP$_1$ | 2.5 (100) |
| cAMP | 1.6 (100) |
| pERK1/2 | 2.9 (100) |

Thomsen et al. *Cell Calcium* 2012

# Agonist signaling bias (example of 12 tested agonists)



IP$_1$ accumulation

- Calcium
- Barium
- Spermine
- Neomycin

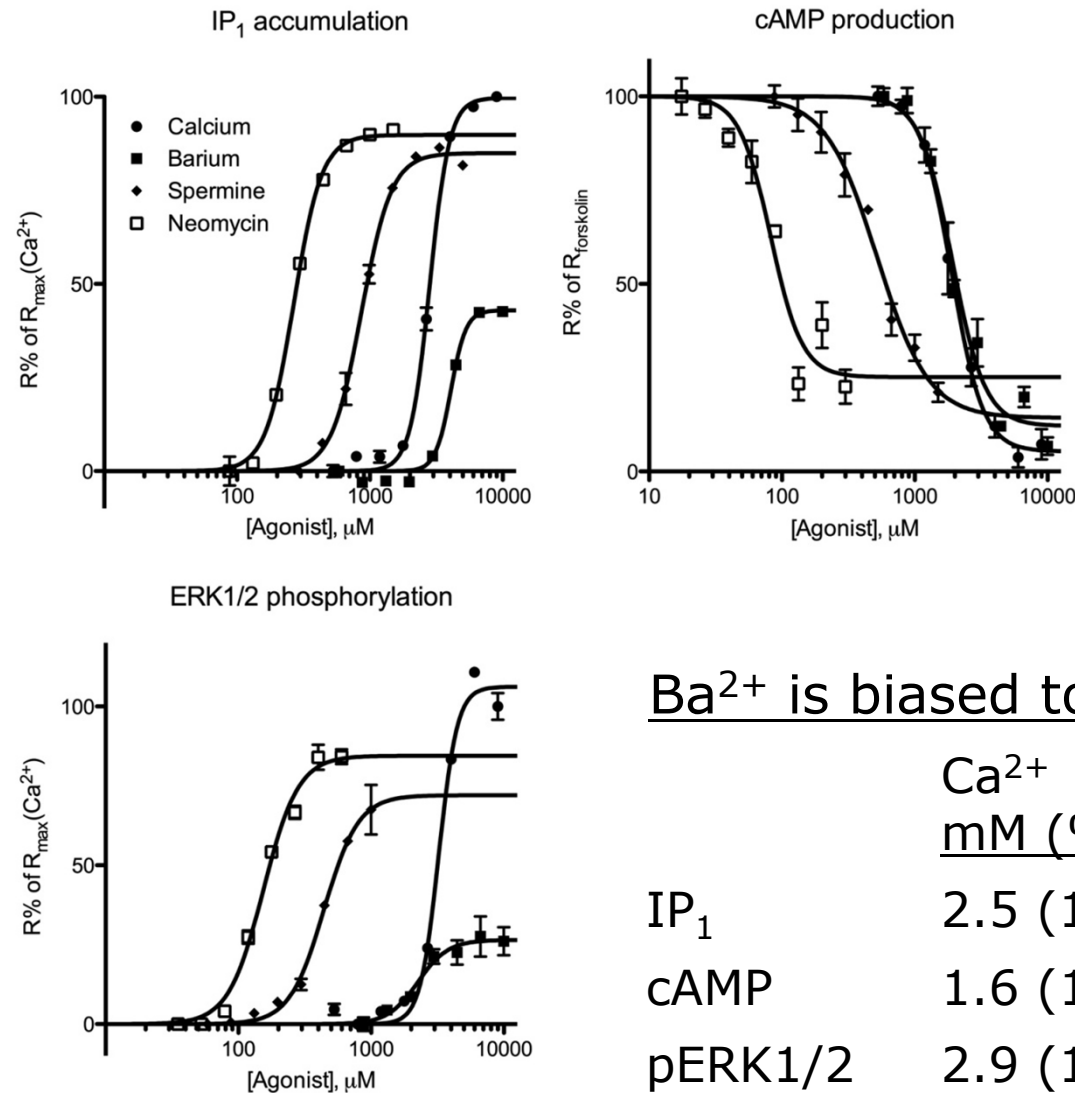R% of R$_{max}$(Ca$^{2+}$)

[Agonist], μM

cAMP production

R% of R$_{forskolin}$

[Agonist], μM

ERK1/2 phosphorylation

R% of R$_{max}$(Ca$^{2+}$)

[Agonist], μM

## Ba$^{2+}$ is biased towards cAMP inhibition

|  | Ca$^{2+}$ mM (%) | Ba$^{2+}$ mM (%) |
|---|---|---|
| IP$_1$ | 2.5 (100) | 3.5 (42) |
| cAMP | 1.6 (100) | 1.7 (84) |
| pERK1/2 | 2.9 (100) | 2.4 (40) |

Thomsen et al. *Cell Calcium* 2012

# Agonist signaling bias (example of 12 tested agonists)



IP$_1$ accumulation

- ● Calcium
- ■ Barium
- ♦ Spermine
- □ Neomycin

R% of R$_{max}$(Ca$^{2+}$)

[Agonist], µM

cAMP production

R% of R$_{forskolin}$

[Agonist], µM

ERK1/2 phosphorylation

R% of R$_{max}$(Ca$^{2+}$)

[Agonist], µM
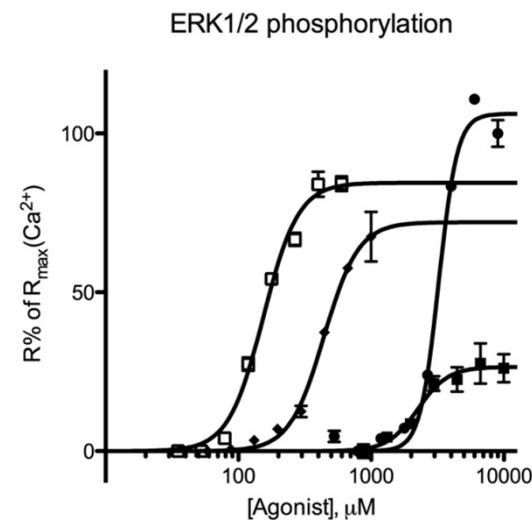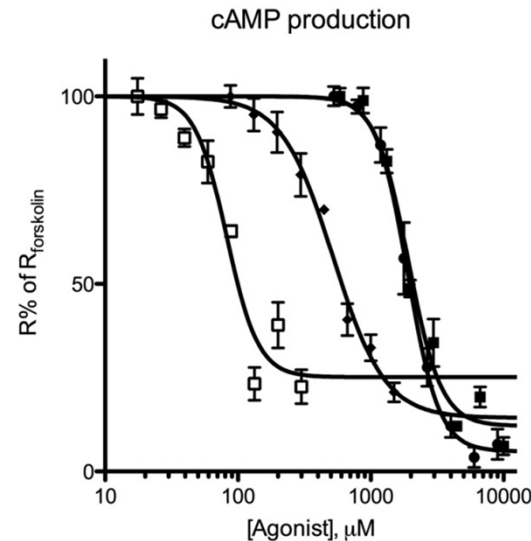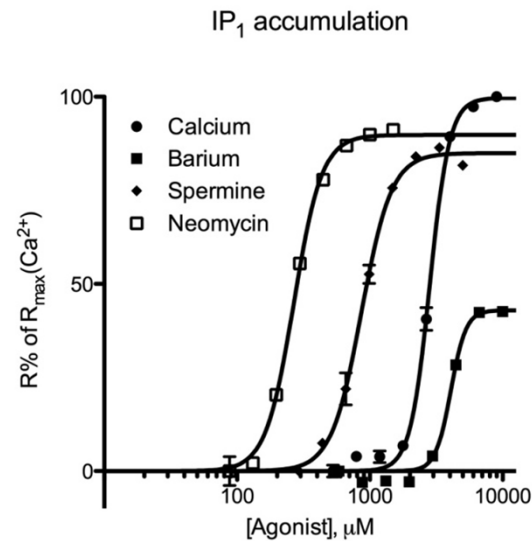
## Spermine is biased towards cAMP inhibition and pERK activation

|          | Ca$^{2+}$ mM (%) | Spermine mM (%) |
|----------|------------------|-----------------|
| IP$_1$   | 2.5 (100)        | 0.82 (87)       |
| cAMP     | 1.6 (100)        | 0.41 (104)      |
| pERK1/2  | 2.9 (100)        | 0.40 (55)       |

Thomsen et al. *Cell Calcium* 2012

# Serum [Ca$^{2+}$] regulation
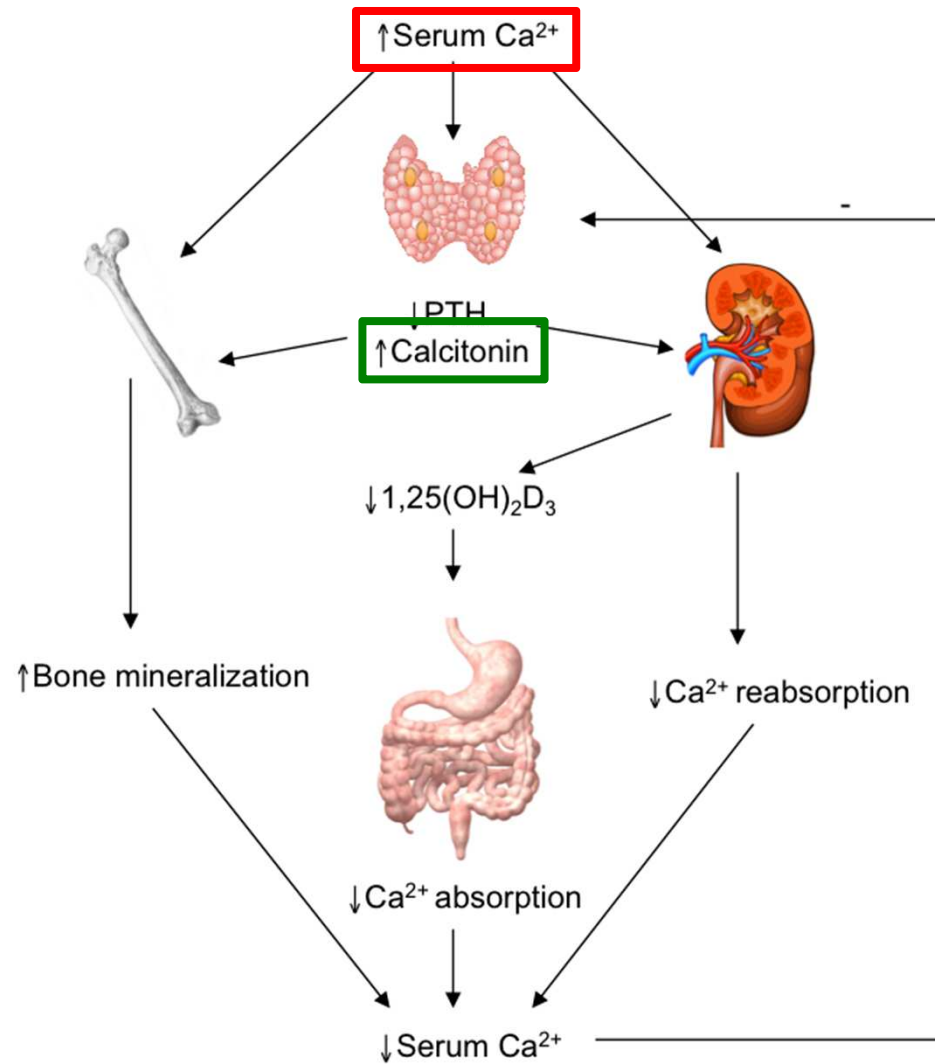
## Rat thyroid carcinoma 6-23 cells express CaSR and release calcitonin

### 6-23 cells
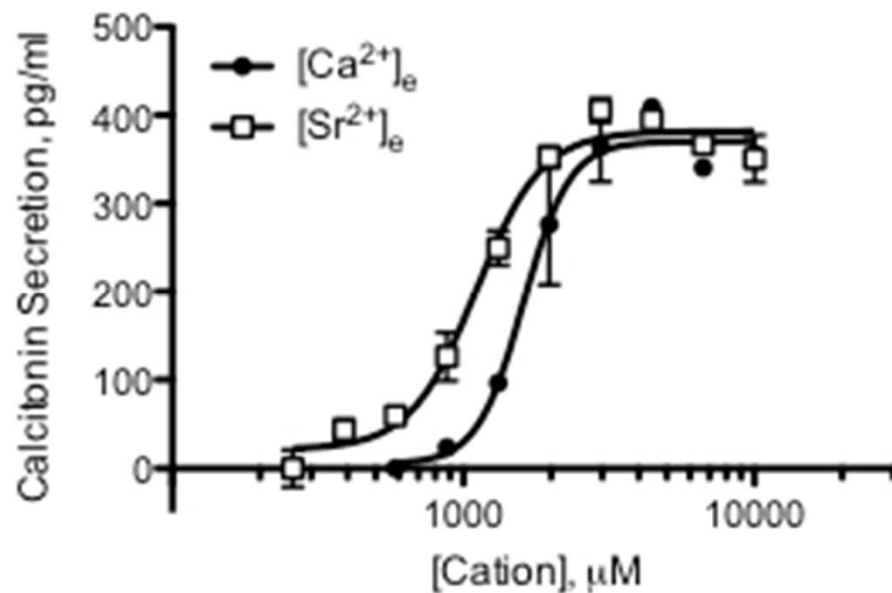Calcitonin release
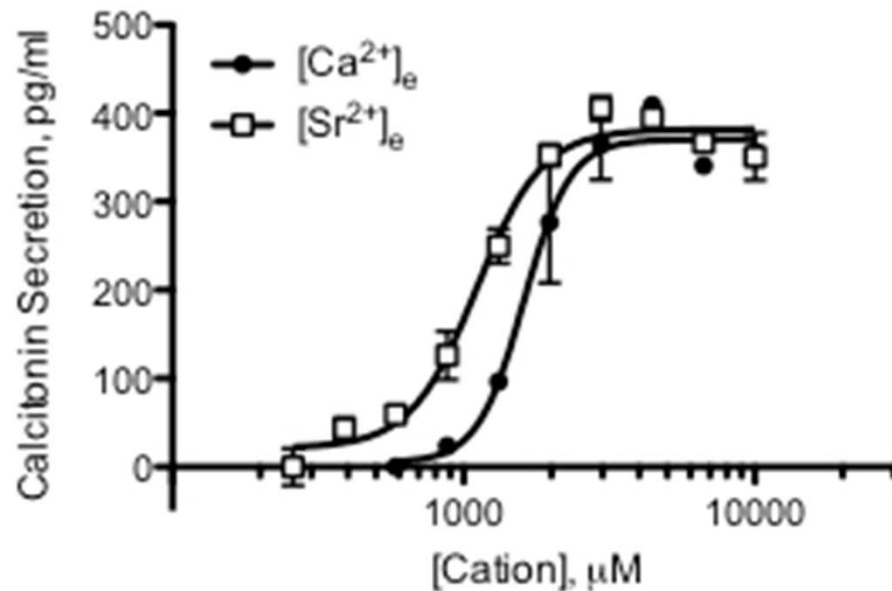
### CaSR-HEK293 cells
$IP_1$ generation

| | Ca$^{2+}$ mM (%) | Sr$^{2+}$ mM (%) |
|---|---|---|
| $IP_1$ | 2.5 (100) | 4.2 (78) |



Thomsen et al. *J. Pharmacol. Exp. Ther.* In press.

Potency rank-order does not correlate between calcitonin release and $IP_1$ generation
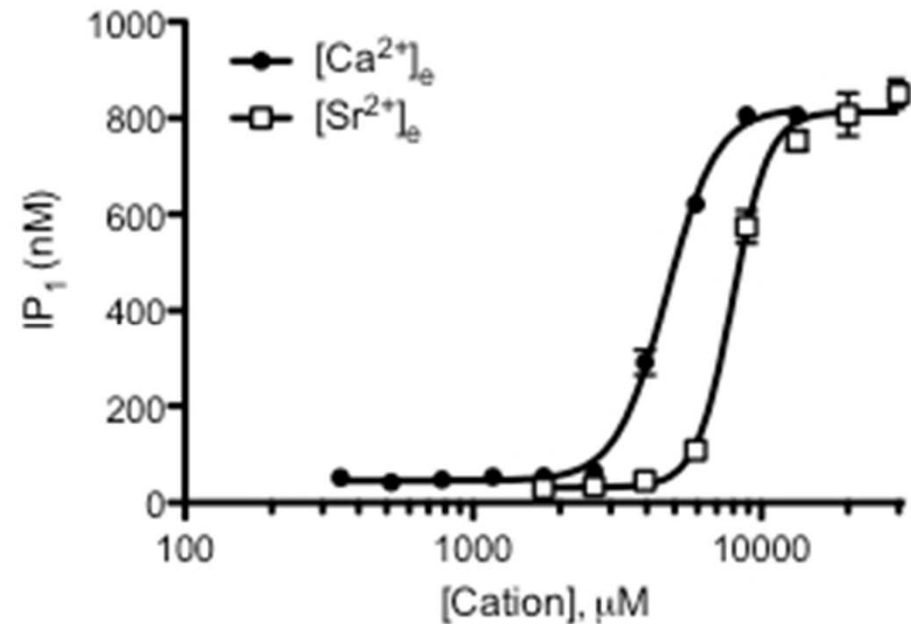
6-23 cells
Calcitonin release

6-23 cells
$IP_1$ generation



$Sr^{2+}$ is more potent than $Ca^{2+}$ in calcitonin release but less potent in $IP_1$ generation indicate involvement of additional pathways and biased agonism.

Thomsen et al. *J. Pharmacol. Exp. Ther.* In press.

## Potency rank-order correlates between calcitonin release and cAMP inhibition and pERK activation

cAMP inhibition

pERK activation
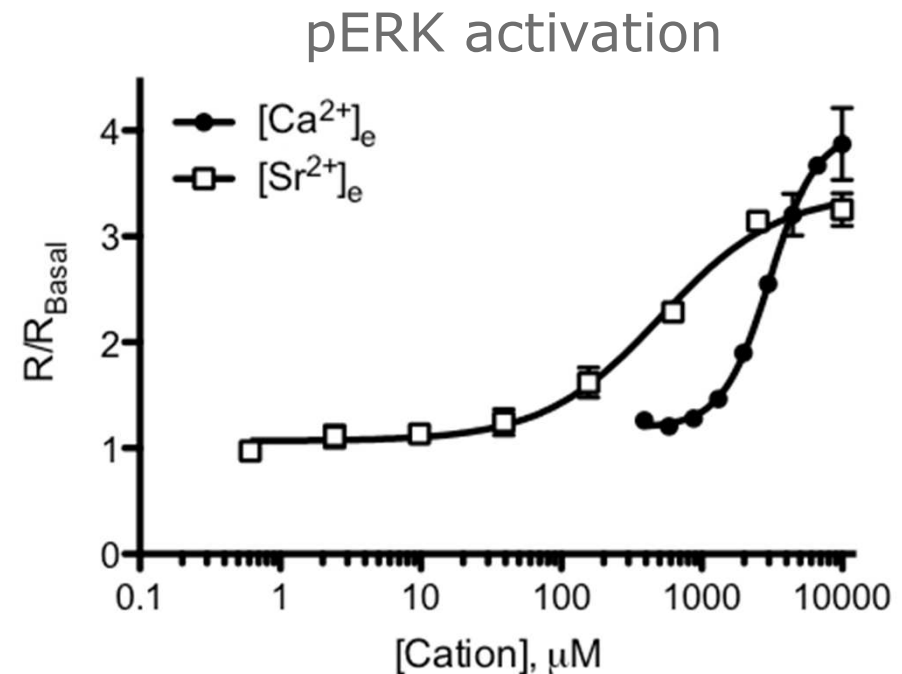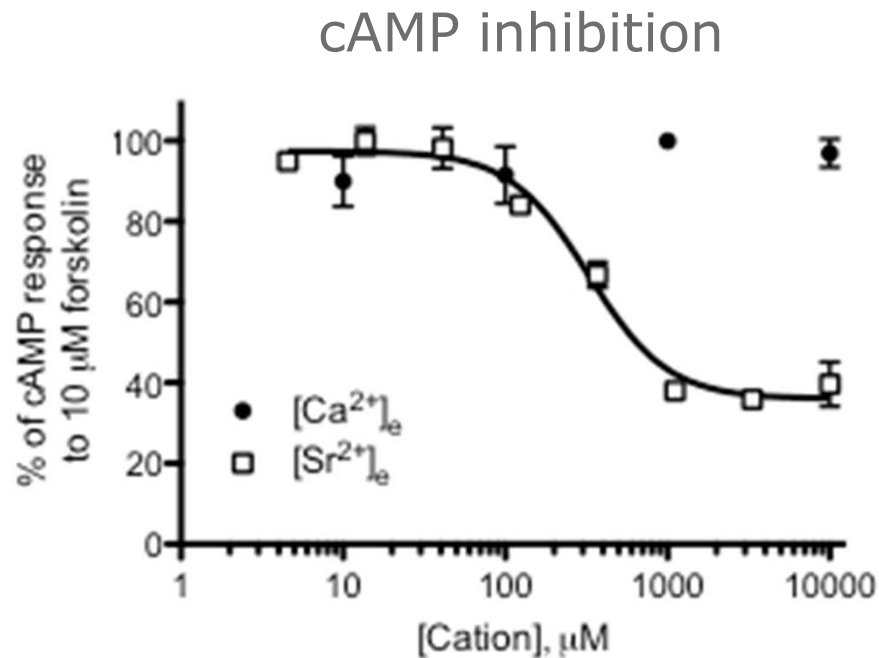
However, pathway inhitors indicate that these pathways are not responsible for the increased potency of $Sr^{2+}$ in mediating calcitonin release.

Thomsen et al. *J. Pharmacol. Exp. Ther.* In press.

## Conclusions

- HTFR assays are effective in measuring the major signaling pathways activated by G protein-coupled receptors
- CaSR agonists have different signaling profiles in cell lines with either recombinant or endogenous receptor expression.
- The active CaSR have multiple conformations with preference for different signaling pathways.
- Development of biased ligands might improve efficacy/side-effect profile for drugs.
- HTRF assays highly efficient for measurement of biased signaling.
  - pERK assay very efficient compared with traditionel Western blot techniques
  - However, like WB, it cannot discriminate which pathways that lead to the observed pERK activation

## Acknowledgements

# HTRF assay of cAMP generation



- 384 well format
- HEK293 cells in suspension
- Plates read on EnVision (PerkinElmer)

Nørskov-Lauritsen et al. *Submitted.*